

The PET Radioligand [¹¹C]MePPEP Binds Reversibly and with High Specific Signal to Cannabinoid CB₁ Receptors in Nonhuman Primate Brain

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The cannabinoid CB₁ receptor is one of the most abundant G protein-coupled receptors in the brain and is a promising target of therapeutic drug development. Success of drug development for neuropsychiatric indications is significantly enhanced with the ability to directly measure spatial and temporal binding of compounds to receptors in central compartments. We assessed the utility of a new positron emission tomography (PET) radioligand to image CB₁ receptors in monkey brain. [¹¹C]MePPEP ((3R,5R)-5-(3-methoxy-phenyl)-3-((R)-1-phenyl-ethylamino)-1-(4-trifluoromethyl-phenyl)-pyrrolidin-2-one) has high CB₁ affinity ($K_b = 0.574 \pm 0.207$ nM) but also moderately high lipophilicity (measured $\text{Log}D_{7.4} = 4.8$). After intravenous injection of [¹¹C]MePPEP, brain activity reached high levels of almost 600% standardized uptake value (SUV) within 10–20 min. The regional uptake was consistent with the distribution of CB₁ receptors, with high radioactivity in striatum and cerebellum and low in thalamus and pons. Injection of pharmacological doses of CB₁-selective agents confirmed that the tracer doses of [¹¹C]MePPEP reversibly labeled CB₁ receptors. Preblockade or displacement with two CB₁ selective agents (ISPB; (4-(3-cyclopentyl-indole-1-sulfonyl)-N-(tetrahydro-pyran-4-ylmethyl)-benzamide) and rimonabant) showed that the majority (>89%) of brain uptake in regions with high receptor densities was specific and reversibly bound to CB₁ receptors in the high binding regions. [¹¹C]MePPEP was rapidly removed from arterial plasma. Regional brain uptake could be quantified as distribution volume relative to the concentration of parent radiotracer in plasma. The P-glycoprotein (P-gp) inhibitor DCPQ ((R)-4-[(1a,6,10b)-1,1-dichloro-1,1a,6,10b-tetrahydrodibenzo[a,e]cyclopropa[c]cyclohepten-6-yl]-[(5-quinolinyl)oxy)methyl]-1-piperazineethanol) did not significantly increase brain uptake of [¹¹C]MePPEP, suggesting it is not a substrate for this efflux transporter at the blood–brain barrier. [¹¹C]MePPEP is a radioligand with high brain uptake, high specific signal to CB₁ receptors, and adequately fast washout from brain that allows quantification with ¹¹C (half-life = 20 min). These promising results in monkey justify studying this radioligand in human subjects. *Neuropsychopharmacology* (2008) **33**, 259–269; doi:10.1038/sj.npp.1301402; published online 28 March 2007

Keywords: cannabinoid; CB₁ receptors; [¹¹C]MePPEP; kinetic analysis; PET

INTRODUCTION

Of the first two described cannabinoid receptor subtypes, the CB₁ receptor is found mainly in the central nervous system, and the CB₂ receptor is found mainly in peripheral immune system cells (Howlett *et al*, 2002). CB₁ receptors are especially abundant in cerebellum, substantia nigra, globus pallidus, hippocampus, and lateral striatum (Herkenham *et al*, 1990). The CB₁ receptor mediates several pharmacological effects and is an active target for therapeutic drug development (Howlett *et al*, 2002; Le Foll and Goldberg, 2005). Cannabinoid receptor ligands may be useful for the

treatment of pain (Walker *et al*, 1999), nausea (Simoneau *et al*, 2001), appetite control (Gelfand and Cannon, 2006; Kirkham and Tucci, 2006), and smoking cessation (Gelfand and Cannon, 2006).

The development of an *in vivo* imaging probe for the CB₁ receptor has been difficult and largely unsuccessful. Similar to the active principal ingredient in marijuana, Δ^9 -tetrahydrocannabinol, most of the proposed radioligands are highly lipophilic. This physiochemical property commonly causes two deficiencies of radioligands. First, plasma protein binding tends to be high (>99%) so that only a small proportion (<1%) is available to cross the blood–brain barrier. Second, brain has a high percentage of lipids, which comprise ~60% of its dry weight (Bennett and Horrobin, 2000). Thus, the majority of a lipophilic tracer that is able to cross the blood–brain barrier is often bound nonspecifically in the brain's abundant lipid environment. Thus, tracers that are too lipophilic tend to have low brain

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Received 28 December 2006; revised 15 February 2007; accepted 19 February 2007

uptake and low ratios of specific to nonspecific binding. These two deficiencies apply to most radiotracers developed to date for the CB₁ receptor. These ligands have been based on the structures of rimonabant (Cosenza *et al*, 2000; Gatley *et al*, 1996; Horti *et al*, 2006; Katoch-Rouse *et al*, 2003; Mathews *et al*, 2000, 2002), cannabinoids (Charalambous *et al*, 1991), and alkylindoles (Gifford *et al*, 2002; Tamagan *et al*, 1999). In contrast to these older agents, [¹¹C]-JHU75528 is a notable exception (see 'Discussion') and has moderate brain uptake and specific signal (Horti *et al*, 2006).

To correct the deficiencies of most previous CB₁ receptor ligands, we recently developed [¹¹C]MePPEP ((3*R*,5*R*)-5-(3-methoxy-phenyl)-3-((*R*)-1-phenyl-ethylamino)-1-(4-trifluoromethyl-phenyl)-pyrrolidin-2-one), which is a CB₁ mixed inverse agonist and antagonist (Figure 1). It has moderately high lipophilicity (measured Log_{D7.4} = 4.8) but still maintains high affinity and selectivity for the CB₁ receptor (K_b at human CB₁ = 0.574 ± 0.207 nM compared to CB₂ = 363 ± 87.4 nM; see 'Results'). The primary purposes of this study were to carefully examine the kinetics of [¹¹C]MePPEP uptake in nonhuman primate brain and to determine whether human studies would be justified. Specifically, we assessed the pharmacological specificity of brain uptake and quantified the imaging data with compartmental modeling and serial arterial measurements of parent radiotracer separated from radiometabolites.

MATERIALS AND METHODS

[γ-³⁵S]GTP Binding Assay Methods

[γ-³⁵S]GTP binding was determined in a 96-well-plate format using a modification of the antibody capture scintillation proximity technique previously described (DeLapp *et al*, 1999). Briefly, 100 μl (0.9 units/well) of membrane preparation from Sf9 cells that ectopically express human CB₁ or CB₂ cannabinoid receptors (PerkinElmer Life and Analytical Sciences, Boston, MA) were incubated for 15 min with 50 μl of test compound diluted in 0.5% fatty acid-free bovine serum albumin (BSA) (Serologicals Corp., Norcross, GA). GDP was added to CB₁ (1 μM final concentration) and CB₂ (0.05 μM final concentration) receptor membranes before incubation. Antagonist/inverse agonist curves were generated in the presence of an EC₈₀ concentration of methanandamide and 0.5% BSA. Following the incubation period, 50 μl [γ-³⁵S]GTP (500 pM final concentration; PerkinElmer Life and Analytical Sciences, Boston, MA) were added to each well and incubated for 35 min. The labeled membranes were solubilized with 0.27% Nonidet P40 for 30 min followed by 30 min of incubation with 20 μl (final dilution of 1:300) of rabbit polyclonal Gα_{i-3} antibody (Covance, Princeton, NJ). Following the antibody incubation, 50 μl (1.25 mg/well) anti-rabbit scintillation proximity assay beads (GE Healthcare, Piscataway, NJ) were added per well and the plates incubated for an additional 60 min. The plates were centrifuged for 10 min at 183g and counted for 1 min per well using a Wallac MicroBeta TriLux scintillation counter (PerkinElmer, Boston, MA). All incubations took place at room temperature in GTP-binding assay buffer (20 mM HEPES, 100 mM NaCl, 5 mM MgCl₂, pH 7.4).

Data were analyzed using a four-parameter logistic-reduced fit with Marquardt-Levenberg algorithm and 200

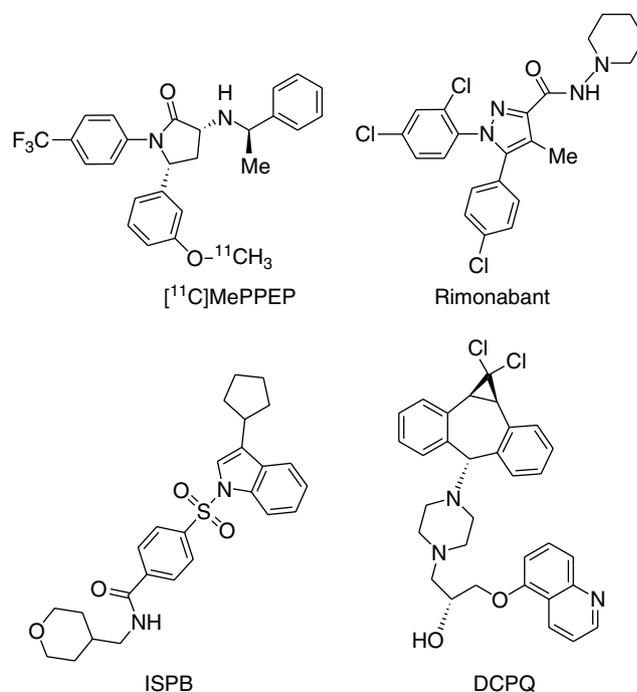


Figure 1 Structures of [¹¹C]MePPEP, rimonabant, ISPB, and DCPQ.

iterations using Activity Base and XLFit2 (IDBS, Emeryville, CA). Background was subtracted from all wells, data were normalized to a full agonist response (1 μM methanandamide), and efficacy was expressed as a percentage of 1 μM methanandamide. Antagonist/inverse agonist percent inhibition data were normalized to results generated with an EC₈₀ concentration of methanandamide. K_b values were determined using a modification of the Cheng-Prusoff relationship: $K_b = IC_{50} / (1 + (\text{agonist}) / EC_{50})$ where IC_{50} is determined from a four parameter fit of displacement curves, $[\text{agonist}] = EC_{80}$ of full agonist (methanandamide), and EC_{50} is determined from a four-parameter fit of the full agonist concentration response curve (Cheng and Prusoff, 1973). Mean EC_{50} and percent inhibition values were calculated as a mean of three independent determinations ± standard error of the mean (SEM).

Broad screening of common cell surface targets was performed by standard radioligand binding techniques through a contract agreement with Cerep (Paris, France). Competitive binding was either not significant or had a K_i value of > 10 μM at the following receptor, ion channel, or enzyme targets: acetylcholinesterase, CCK1, CCK2, EDG-2, Cox1, Cox2, adenosine A1, adenosine A3, adrenergic alpha1, adrenergic alpha2, angiotensin AT1, adrenergic β1, adrenergic β2, benzodiazepine site, Ca²⁺ channel (verapamil site), dopamine D1, dopamine D2S, GABA, Ghrelin, histamine H1, histamine H2, K⁺ channel (HERG), non-selective muscarinic, NE transporter, NK1, NK2, nonselective opiate, nonselective 5-HT.

Preparation and Analysis of [¹¹C]MePPEP

[¹¹C]Iodomethane produced by the GE PETtrace Microlab was delivered with helium carrier gas at a flow rate of 10 ml/min into a 2 ml stainless loop containing 1 mg phenolic precursor (22 nM) and 1 equivalent tetrabutylammonium

hydroxide in 80 μ l dimethyl formamide. The reaction proceeded for 5 min, and the entire contents of the loop were injected onto the HPLC column. The labeled product was purified on C₁₈ column (Phenomenex luna, 10 \times 250 mm) with isocratic mobile phase (70:30 = acetonitrile/0.1 M ammonium formate, pH adjusted to 7.5 with 1 M ammonium hydroxide in water) at the flow rate of 6 ml/min. Product eluted at 12 min and precursor at 6.3 min. Solvents were removed by rotary evaporation at 80°C. The product was formulated with 10 ml saline containing 20 mg Tween 80 within 35 min of the end of bombardment.

Measurement of LogD_{7.4}

[¹¹C]MePPEP was dissolved in 0.9% NaCl/10% ethanol to a specific concentration of about 10 μ Ci/ μ l. The radiochemical purity of this preparation was greater than 99%. Six determinations of LogD_{7.4} were carried out at room temperature in n-octanol and 0.15 M phosphate buffer (pH 7.4), as previously described (Zoghbi *et al*, 1993), but with correction of the radioactivity associated with parent radioligand in the aqueous phase after radio-HPLC. The percent error of the γ -countermeasurements of the samples with the least counts was 4.6 \pm 0.3%.

Animals

All animal experiments complied with the Guide for Care and Use of Laboratory Animals and were approved by the NIMH Animal Care and Use Committee. We used six male rhesus monkeys (weight: 12.6 \pm 2.0 kg, with these and subsequent data expressed as mean \pm SD), four of which underwent arterial blood sampling. Animals were immobilized with ketamine (10 mg/kg i.m.) for intubation. After transportation to the positron emission tomography (PET) suite, the animals were placed under isoflurane anesthesia (1–2%), and the head was immobilized in a stereotactic frame. To minimize the effects of ketamine, scans started at least 90 min after ketamine administration.

We used the high resolution research tomograph (HRRT; Siemens/CPS, Knoxville, TN, USA), which has a reconstructed resolution of 2.5 mm at full-width half-maximum in all directions in 3D mode. A transmission scan was initially performed for attenuation correction. [¹¹C]MePPEP was administered over \sim 60 s. Scans of 120 min (33 frames) were acquired in all studies, whereas longer scans (39 frames over 150 min) were acquired in one monkey to evaluate the effect of the scan duration on the plasma compartmental analysis.

Arterial blood was collected in heparin-treated syringes from four monkeys at 15, 30, 45, 60, 75, 90, 105 s and 2, 3, 5, 10, 30, 60, 75, 90, 120 min after injection. The parent tracer, separated from radiometabolites, was measured as previously described (Zoghbi *et al*, 2005). We also measured the plasma-free fraction (*f*₁) of [¹¹C]MePPEP in two monkeys (E and F; Table 1). Plasma-free fraction was measured by ultrafiltration through Centrifree membrane filters (Amicon Division, W.R. Grace and Co., Danvers, MA, USA) (Gandelman *et al*, 1994) in two monkeys. Adequate radioactivity was used so that the percentage of counting error in the least radioactive samples ranged between 3.0 \pm 0.3 and 5.3 \pm 1.3% at one standard deviation.

Receptor preblocking studies were performed in two monkeys using rimonabant (previously called SR141716A) and ISPB (4-(3-cyclopentyl-indole-1-sulfonyl)-N-(tetrahydro-pyran-4-ylmethyl)-benzamide) (Figure 1), both of which are selective for the CB₁ receptor and have mixed inverse agonist and antagonist properties. ISPB (1.0 mg/kg i.v.) or rimonabant (3.0 mg/kg i.v.) were injected 10 min before [¹¹C]MePPEP (Table 1, monkeys A and B). A displacement study was also carried out for one monkey, in which nonradioactive ISPB (1.5 mg/kg i.v.) was injected 60 min after [¹¹C]MePPEP (Table 1, monkey A).

In this displacement study, data of the initial 9 min were not acquired because of a scanner problem. These studies of preblocking were performed in the afternoon, following the baseline scan of the same animal in the morning.

Each monkey had a T1-weighted magnetic resonance imaging (MRI) scan on a GE Sigma 1.5 T scanner (SPGR, TR/TE/flip angle = 13.1/5.8 ms/45°, 0.4 \times 0.4 \times 1.5 mm with coronal acquisitions on a 256 \times 256 \times 60 matrix).

Image Analysis

Regional brain radioactivity was obtained with a modified template-based method that consisted of three steps (Yasuno *et al*, 2002). The first step involved the spatial transformation of the regional template from a model MRI to an individual animal's MRI. The second step was the manual correction of the error of transformed volumes of interest with reference to the animal's MRI (Figure 2). Finally, corrected template volumes were linearly transformed to the PET images using the parameters obtained from the coregistration of the MRI. Seven template volumes were defined: cerebellum (2.1 cm³), prefrontal cortex (8.8 cm³), lateral temporal cortex (5.9 cm³), medial temporal region including the hippocampus (3.1 cm³), striatum (2.7 cm³), thalamus (0.9 cm³), and pons (0.8 cm³).

Table 1 PET Studies Performed with [¹¹C]MePPEP

Monkey	Arterial blood sampling	Plasma-free fraction	Scan duration (min)	Condition
A	+	–	150/120 ^a	Baseline and preblockade and displacement with ISPB
B	+	–	120	Baseline and preblockade with rimonabant
C	+	–	120	Baseline
D	+	–	120	Baseline and pretreatment with DCPQ (8.0 mg/kg)
E	–	+	120	Baseline and pretreatment with DCPQ (8.0 mg/kg)
F	–	+	120	Baseline and pretreatment with DCPQ (8.0 mg/kg)

^a150 min duration for the baseline and 120 min for preblockade.

Estimation of Distribution Volume with Arterial Input Function

The brain and plasma data were analyzed with one- and two-tissue compartment models using rate constants (K_1 , k_2 , k_3 , and k_4) as previously described (Laruelle *et al*, 1994). Total distribution volume V_T measures specific receptor binding, nonspecific binding, and free radiotracer. The nondisplaceable component V_{ND} is the sum of the latter two—nonspecific binding plus free radiotracer.

For the one-tissue compartment model:

$$V_T = \frac{K_1}{k_2}$$

For the two-compartment model, V_T is the sum of the distribution volumes of nondisplaceable uptake (V_{ND}) and specific binding (V_S).

$$\begin{aligned} V_{ND} &= \frac{K_1}{k_2} \\ V_S &= \frac{K_1 k_3}{k_2 k_4} \\ V_T &= V_{ND} + V_S \\ &= \left(\frac{K_1}{k_2}\right) \left(1 + \frac{k_3}{k_4}\right) \end{aligned}$$

The kinetic analyses were performed using the software program PMOD 2.75 (Burger *et al*, 1998).

Time-Stability of Parameter Estimates

The relationship between parameter estimates and the duration of the scan was analyzed in one monkey with increasingly truncated data sets from 0–150 to 0–60 min (Table 1, monkey A). The estimated parameters were compared with the reference values obtained with the complete 150 min data set. For the time activity data from the mean of all measured regions, specific binding was

estimated in the two-tissue compartmental model. The solution was considered stable after time t if the result was within 10% of that from the analysis of the entire data set (ie 0–150 min).

Effect of P-gp on Brain Uptake and Peripheral Disposition of [¹¹C]MePPEP

We compared the uptake of [¹¹C]MePPEP in three studies (Table 1, monkey D, E, and F) with and without the administration of 8 mg/kg i.v. of the P-glycoprotein (P-gp) inhibitor DCPQ ((*R*)-4-[(1*a*,6,10*b*)-1,1-dichloro-1,1*a*,6,10*b*-tetrahydrodibenzo[*a,e*]cyclopropa[*c*]cyclohepten-6-yl]-[(5-quinolinyl)oxy)methyl]-1-piperazineethanol; Figure 1). DCPQ was previously reported as compound 14*b* (Pfister *et al*, 1995).

Under terms of the Material Transfer Agreement with Xenova Group, Ltd (UK) we could not use their better known P-gp inhibitor tariquidar, previously called XR9576 (Roe *et al*, 1999) with proprietary compounds in the current collaboration. Instead, we compared the efficacy of tariquidar and DCPQ to enhance brain uptake of [¹¹C]lopramide, which is an avid substrate for P-gp (Sadeque *et al*, 2000). DCPQ and tariquidar (each at 8 mg/kg i.v.) was administered 30 min before [¹¹C]loperamide and increased rhesus brain activity to 415 and 316%, respectively.

Statistical Analysis

Goodness-of-fit by nonlinear least-squares analysis was evaluated using the model selection criterion (MSC), which is a modification of the Akaike information criterion (Akaike, 1974). MSC gives greater values for better fitting. Goodness-of-fit by one- and two-tissue compartmental models was compared with F statistics (Hawkins *et al*, 1986).

The standard error of nonlinear least-squares estimation for rate constants was given by the diagonal of the covariance matrix (Carson, 1986) and expressed as a percentage of the rate constants (coefficient of variation, %COV). In addition, %COV of total distribution volume was calculated from the covariance matrix using the generalized form of error propagation equation (Bevington and Robinson, 2003), where correlations among parameters were taken into account.

RESULTS

In Vitro Activity of MePPEP

In membranes prepared from Sf9 cells ectopically expressing the human CB₁ or CB₂ receptors, *in vitro* functional binding activity of nonlabeled MePPEP was determined using an antibody capture scintillation proximity [³⁵S]GTP binding assay and compared to rimonabant (previously called SR141716A). MePPEP inhibited functional [³⁵S]GTP binding at the human CB₁ receptor with high potency ($K_b = 0.574 \pm 0.207$ nM) compared to rimonabant ($K_b = 5.96 \pm 0.209$ nM). MePPEP was significantly less potent at the human CB₂ receptor ($K_b = 363 \pm 77.9$ nM for MePPEP and $K_b > 10\,000$ nM for rimonabant at CB₂).

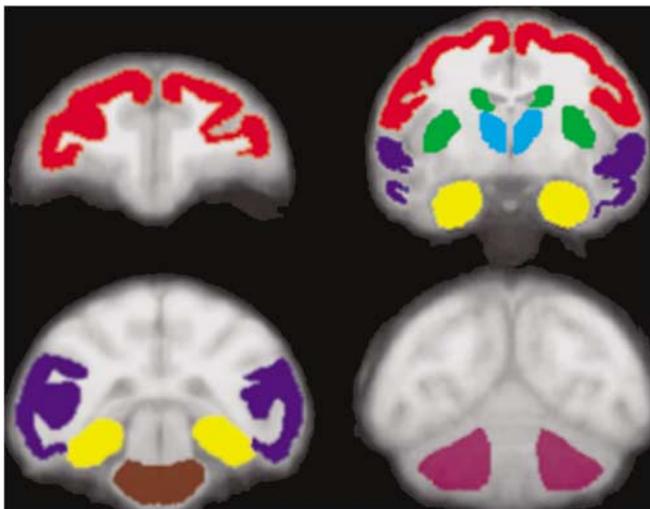


Figure 2 Volume of interest template defined on the model MRI. Red, prefrontal cortex; dark blue, lateral temporal cortex; yellow, medial temporal region (including hippocampus); green, striatum; light blue, thalamus; brown, pons; purple, cerebellum.

Preparation of [¹¹C]MePPEP

The radioligand was prepared within 35 min of the end of bombardment and had yield = $2.5 \pm 1.1\%$, radiochemical purity = $100 \pm 0\%$, chemical purity = $99.9 \pm 0\%$, and specific activity at time of injection = 78.1 ± 54.9 GBq/ μ mol ($n = 12$ batches). The mean injected activity was 232 ± 70 MBq.

The $\text{Log}D_{7.4}$ of [¹¹C]MePPEP was 4.8 ± 0.3 ($n = 6$), measured in octanol/saline at pH 7.4.

Pharmacological Effects in Nonhuman Primates

In PET scans, the injected mass dose of [¹¹C]MePPEP was 0.11 ± 0.06 μ g/kg. In all baseline and blocking experiments using ISPB (1.0–1.5 mg/kg) and rimonabant (3.0 mg/kg), the differences between pre- and postinjection vital signs were: <17 mm Hg for systemic blood pressure, <17 min⁻¹ for pulse, <3 min⁻¹ for respiratory rate, and <0.1°C for temperature.

Brain Uptake of [¹¹C]MePPEP

Following injection of [¹¹C]MePPEP, brain activity increased to high levels (almost 600% standardized uptake (SUV) in cerebellum), with peak values within 10–20 min. The distribution of activity was consistent with binding to CB₁ receptors, with high levels in striatum and cerebellum and low in thalamus and pons (Figures 3 and 4a). The brain uptake of [¹¹C]MePPEP was preblocked with receptor saturating doses of nonradioactive ISPB (1.0 mg/kg i.v.) or rimonabant (3.0 mg/kg i.v.) (Figure 4b). In addition, brain uptake was rapidly displaced by nonradioactive ISPB (1.5 mg/kg i.v.) at 60 min after tracer injection (Figure 4c). In summary, brain uptake of [¹¹C]MePPEP was consistent with reversible labeling of CB₁ receptors, showing an appropriate regional distribution, pharmacological selectivity, and saturability.

Arterial Plasma Analysis

The radioligand was quickly metabolized and represented 83 ± 12 , 53 ± 17 , 21 ± 7 , and $14 \pm 5\%$ of total plasma activity at 5, 10, 30, and 60 min, respectively, in all the four monkey studies. [¹¹C]MePPEP was rapidly removed from arterial plasma, with a clearance rate of 361 ± 40 ml/min. Plasma activity of unchanged [¹¹C]MePPEP peaked at ~ 1 min and decreased to $3.1 \pm 0.9\%$ of the peak by 10 min.

Monkey arterial plasma samples had four radioactive peaks. A sample radiochromatogram of plasma at 10 min (Figure 5a) was composed of polar peak 1 (34.4%); two intermediate peaks, 2 (5.4%) and 3 (1.2%); and parent radioligand 4 (59%). The plasma parent composition declined rapidly to about 18% as early as 30 min after the injection of [¹¹C]MePPEP. Radiometabolite 1 was the major radioactive component at the end of the study (Figure 5b).

Nonlinear Least-Squares Compartmental Analyses

We used brain activity and serial arterial plasma measurements of parent radiotracer to estimate distribution volume in brain using four scanning sessions in four different animals (monkeys A–D; Table 1). Convergence was achieved in the baseline scans using both one- and

two-tissue compartmental model analyses of 120 min scan data, but the two-tissue compartmental model provided significantly better fits (Tables 2 and 3; Figure 6). MSC values were higher for two-tissue (3.4 ± 0.7) than for one-tissue (2.2 ± 0.7) compartmental models, and this difference was significant by *F*-test in all regions of all animals ($p < 0.001$).

In two-tissue compartmental model, the standard errors of individual rate constants showed poor identifiability with COV values >10% in most regions. However, V_S and V_T were relatively well identified in each model, with COV values <8%. Although values of V_T obtained with the one- and two-tissue compartment models correlated well ($r = 0.99$), the one-tissue model apparently underestimated V_T by an average of 12% (range 426%), which may have been caused by its relatively poor goodness of fit.

We also calculated distribution volume in two scanning sessions (monkeys A and B, Table 1) with preblockade of rimonabant or ISPB and compared them to the baseline V_T of same animals. In these two studies, the mean regional value of nondisplaceable uptake (V_{ND}) in high binding regions (ie all except thalamus and pons) was about 11% of V_T (Table 4). Thus, we estimate that 89% of total brain uptake was associated with CB₁ receptors.

Effect of P-gp on Brain Uptake and Peripheral Disposition of [¹¹C]MePPEP

DCPQ (8 mg/kg i.v.) significantly increased the uptake of [¹¹C]MePPEP in all brain regions (Figure 7a). In contrast, DCPQ had no significant effect on the clearance of [¹¹C]MePPEP measured in arterial plasma corrected for radiometabolites (Figure 7b). In the three animals tested, DCPQ caused a $\sim 17\%$ increase of the mean area under the brain curves of all six regions, calculated from 0 to 120 min (Table 5). These results showed an apparent effect of P-gp blockade—increased brain uptake in the absence of noticeable changes of total tracer concentration in plasma. However, we also examined the effect of DCPQ on f_1 of tracer. DCPQ increased f_1 in each animal, with a mean increase of 10% (Table 5).

Correction of the area under the curve for change in f_1 suggested that DCPQ caused only a 6% effect on brain activity independent of its peripheral effects. That is, the corrected ratio under the brain activity curves between baseline and DCPQ was 1.06 (= 1.17/1.10). An increase of 6% with P-gp blockade is quite small relative to tracers that are known to be efflux pump substrates, which are elevated 400–1300% with genetic inactivation of P-gp (Schinkel *et al*, 1996). This 6% increase may also be insignificant in light of possible measurement errors for the small absolute values of f_1 (0.04–0.07%; Table 5).

DISCUSSION

The uptake of [¹¹C]MePPEP in nonhuman primate brain demonstrated reversible labeling of CB₁ receptors. Brain uptake showed appropriate pharmacological properties, as it could be displaced and/or blocked by agents from two different chemical classes, ISPB and rimonabant. The clearance of [¹¹C]MePPEP in monkey was adequately fast

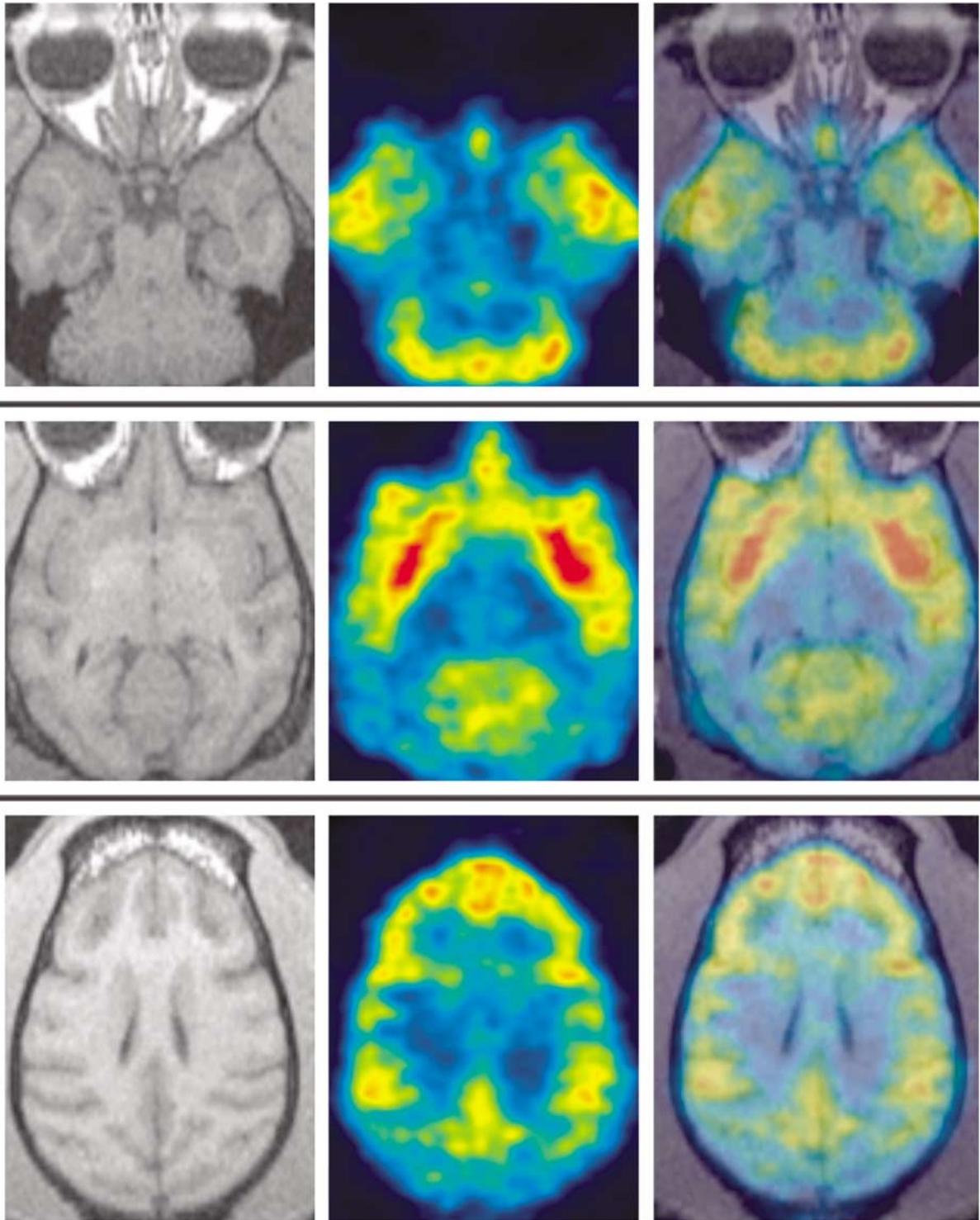


Figure 3 PET summation image in rhesus brain. PET images (middle column) were summed from 60 to 120 min. The corresponding MR images are on left column, and the fused PET and MR images are on right column. Upper row: slice including cerebellum and medial and lateral temporal cortex. Middle row: slice including striatum, thalamus, and lateral temporal cortex. Lower row: slice including prefrontal cortex.

to generate peak brain uptake within 20 min, which is well within the effective imaging time of ¹¹C that has a half-life of 20 min.

The P-gp inhibitor DCPQ (8 mg/kg i.v.) increased brain uptake by ~17%, with insignificant change in the parent tracer in plasma (ie the amount of tracer exposed to brain; Figure 7). However, DCPQ increased f_1 by ~10%, and the

corrected ratio of area under the curve between baseline and DCPQ was 1.06 (= 1.17/1.10), which may merely reflect errors in measurement of the low values of f_1 (Table 5). In addition, a 17% increase is small compared to >400% found for agents that are avid substrates for P-gp (Schinkel *et al*, 1996). Thus, our data suggest that [¹¹C]MePPEP is a relatively insignificant substrate for P-gp in monkey brain.

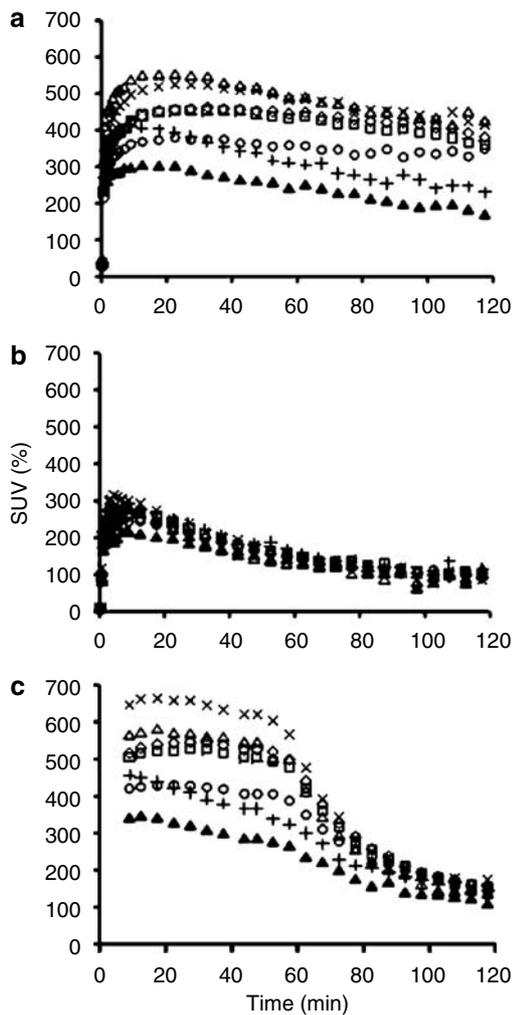


Figure 4 (a) Time course of regional brain activities after injection of [¹¹C]MePPEP. Activity is shown as %SUV which normalizes for injected dose and body weight. %SUV = (% injected activity/cm³ tissue)(g body weight) cerebellum, Δ; striatum, ×; prefrontal cortex, ◇; lateral temporal cortex, □; medial temporal region, ○; thalamus, +; and pons, ▲. (b) Receptors were blocked by pretreatment with ISPB (1.0 mg/kg i.v.) before injection of [¹¹C]MePPEP. (c) Radioligand was displaced by ISPB (1.5 mg/kg i.v.) injected 60 min after [¹¹C]MePPEP. Data from the initial 9 min are missing because of scanner malfunction.

Advantages of [¹¹C]MePPEP

What are the relative advantages and limitations of [¹¹C]MePPEP as a probe for central CB₁ receptors? The advantages include:

- (1) [¹¹C]MePPEP has high brain uptake, with peak levels of almost 600% SUV. Such high uptake means that brain activity can be more accurately quantified and/or the injected activity can be reduced. For comparison, the peak uptake of two other PET radiotracers in monkey is 300% SUV in striatum for the dopamine D_{2/3} receptor probe [¹¹C]raclopride (Nyberg *et al*, 2002) and 500% SUV in medial temporal region for the 5-HT_{1A} receptor probe [¹¹C](R)-(-)-RWAY (Yasuno *et al*, 2006). The high brain uptake of [¹¹C]MePPEP reflected, in part, CB₁ receptor density, which is the highest of any G

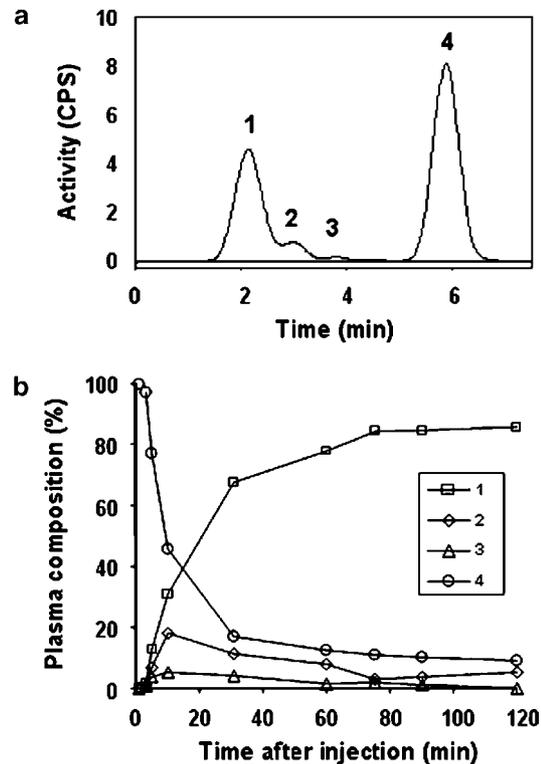


Figure 5 (a) Radiochromatogram of a plasma sample obtained at 10 min after intravenous injection of [¹¹C]MePPEP. Peak 1 was the most polar radiometabolite and eluted in the void volume of the column. Peak 4 was the parent radioligand. (b) Representative plasma composition over time of [¹¹C]MePPEP (4) and its three radiometabolites (1, 2, and 3).

protein-coupled receptor in brain (Begg *et al*, 2005). The density (B_{max}) of CB₁ receptors in cerebellum in rat is 1752 fmol/mg protein (Hirst *et al*, 1996), compared to dopamine D₂ receptors in striatum (B_{max} = 247 fmol/mg protein) (Dewar *et al*, 1989) and 5-HT_{1A} receptors in hippocampal region (312 fmol/mg protein) (Khawaja *et al*, 1995).

- (2) [¹¹C]MePPEP has a high percentage of specific binding. We performed compartmental analysis in two different preblocked conditions. The results suggested at least 89% of uptake was specific in high binding regions. If we had administered a dose that completely blocked CB₁ receptors, then the estimate of specific uptake would have been even higher. In summary, both baseline and preblocked analysis showed that the vast majority (>89%) of brain uptake is bound to CB₁ receptors (ie specific binding).
- (3) The rapid plasma clearance of [¹¹C]MePPEP is important to measure distribution volume. As a general rule, quantitation of brain uptake requires imaging before and slightly after the time of peak uptake. Receptor density and plasma clearance have opposite effects on the time of peak uptake. That is, higher densities of receptors cause later times of peak uptake, as these regions can bind more ligand at equilibrium. In contrast, faster plasma clearance causes an earlier peak in all organs, because the ligand is removed from the body more rapidly. As CB₁ receptors are present at such high densities in brain, the clearance of a useful radioligand must be rapid so that the peak can occur

Table 2 Parameter Estimates (Mean ± SD) Obtained from a Two-Tissue Compartment Model

Region	K_1 (ml min ⁻¹ cm ⁻³)	k_2 (min ⁻¹)	k_3 (min ⁻¹)	k_4 (min ⁻¹)	V_s (ml cm ⁻³)	V_T (ml cm ⁻³)	MSC
	%COV	%COV	%COV	%COV	%COV	%COV	
Prefrontal cortex	0.22 ± 0.08	0.70 ± 0.21	1.20 ± 0.44	0.01 ± 0.004	53.5 ± 24.7	53.8 ± 24.9	4.8 ± 0.6
	6.3 ± 5.4	22.6 ± 18.4	7.5 ± 6.0	13.6 ± 9.6	5.2 ± 4.2	5.2 ± 4.2	
Lateral temporal Cortex	0.24 ± 0.09	0.71 ± 0.25	1.13 ± 0.38	0.01 ± 0.004	42.7 ± 20.8	43.1 ± 20.9	5.1 ± 0.3
	4.9 ± 2.6	14.7 ± 10.0	7.1 ± 3.3	8.8 ± 4.2	2.8 ± 1.2	2.7 ± 1.2	
Medial temporal Region	0.23 ± 0.11	0.62 ± 0.15	0.67 ± 0.07	0.01 ± 0.003	36.4 ± 15.8	36.8 ± 15.9	3.8 ± 1.3
	11.6 ± 8.2	31.4 ± 15.3	13.7 ± 5.2	11.7 ± 3.6	5.4 ± 2.5	5.4 ± 2.5	
Striatum	0.27 ± 0.15	0.59 ± 0.30	1.02 ± 0.40	0.01 ± 0.004	46.9 ± 19.1	47.4 ± 19.3	4.3 ± 0.3
	8.0 ± 2.3	45.1 ± 34.2	25.0 ± 20.6	15.9 ± 15.0	4.8 ± 4.1	4.8 ± 4.2	
Thalamus	0.23 ± 0.09	0.54 ± 0.40	0.63 ± 0.27	0.02 ± 0.004	16.2 ± 8.0	16.7 ± 8.3	3.1 ± 0.5
	8.4 ± 3.2	34.8 ± 25.3	21.3 ± 15.3	12.1 ± 7.0	3.1 ± 1.0	2.8 ± 0.6	
Cerebellum	0.29 ± 0.11	0.54 ± 0.52	0.68 ± 0.53	0.01 ± 0.002	32.2 ± 12.5	33.4 ± 12.5	3.5 ± 0.6
	10.3 ± 8.6	42.0 ± 13.2	24.9 ± 10.4	14.9 ± 4.8	4.2 ± 2.5	4.1 ± 2.5	
Pons	0.28 ± 0.11	1.86 ± 1.14	0.97 ± 0.21	0.01 ± 0.003	16.2 ± 7.4	16.4 ± 7.5	3.1 ± 0.9
	15.8 ± 1.2	37.4 ± 13.6	16.5 ± 11.2	11.7 ± 7.5	4.6 ± 4.3	4.6 ± 4.3	

COV, coefficient of variation; MSC, model selection criteria; SD, standard deviation; V_s , specific binding potential; V_T , total distribution volume.

The values on the first of two lines for each region are the mean ± SD of the parameter determined in three scanning studies.

The values on the second line are the identifiability of the parameter (called 'standard error' of the compartmental fit) and are expressed as %COV. For example, K_1 in prefrontal cortex was 0.22 ± 0.08 ml min⁻¹ cm⁻³ and had a mean ± SD identifiability of 6.3 ± 5.4% of that value.

Table 3 Parameter Estimates (Mean ± SD) Obtained from a One-Tissue Compartment Model

Region	K_1 (ml min ⁻¹ cm ⁻³)	k_2 (min ⁻¹)	V_T (ml cm ⁻³)	MSC
	%COV	%COV	%COV	
Prefrontal cortex	0.14 ± 0.04	0.003 ± 0.001	46.0 ± 18.8	3.5 ± 0.3
	1.3 ± 0.3	7.8 ± 3.7	6.8 ± 3.4	
Lateral temporal cortex	0.15 ± 0.04	0.004 ± 0.001	37.9 ± 16.2	3.4 ± 0.4
	1.3 ± 0.3	6.1 ± 2.2	5.1 ± 2.0	
Medial temporal region	0.13 ± 0.05	0.004 ± 0.001	29.5 ± 12.3	1.9 ± 0.4
	2.3 ± 0.01	10.0 ± 1.9	8.3 ± 1.9	
Striatum	0.18 ± 0.07	0.004 ± 0.002	42.1 ± 15.7	3.1 ± 0.4
	1.4 ± 0.3	6.7 ± 2.9	5.6 ± 2.7	
Thalamus	0.1 ± 0.05	0.010 ± 0.002	15.2 ± 7.0	1.9 ± 0.2
	2.31 ± 0.21	5.7 ± 0.7	4.2 ± 0.6	
Cerebellum	0.2 ± 0.06	0.006 ± 0.001	29.4 ± 11.1	2.3 ± 0.2
	1.9 ± 0.2	6.0 ± 1.0	4.7 ± 0.8	
Pons	0.11 ± 0.04	0.008 ± 0.003	14.7 ± 6.6	1.5 ± 0.2
	2.7 ± 0.3	8.3 ± 2.7	6.4 ± 1.9	

COV, coefficient of variation; MSC, model selection criteria; SD, standard deviation; V_T , total distribution volume.

See legend of Table 1 for meaning of values on upper and lower lines for each region.

within the useful imaging time of the radionuclide. Thus, the rapid plasma clearance of [¹¹C]MePPEP (see 'Results') is critical to quantify distribution volume, which is proportional to receptor density.

- (4) [¹¹C]MePPEP is relatively easily prepared with high purity (>99%) and in reasonable overall yield (3%) using a commercially available ¹¹C-methylation apparatus.
- (5) The short half-life of ¹¹C allows two or more scanning studies to be completed in a single subject in 1 day. Such a repeated scanning schedule can accommodate phar-

macological challenges or measurements of receptor occupancy by CB₁ receptor-selective medications.

Limitation of [¹¹C]MePPEP

The limitations of [¹¹C]MePPEP and imaging the CB₁ receptor in general include:

- (1) The plasma-free fraction f_1 of [¹¹C]MePPEP was quite low (0.04–0.07%) and difficult to measure accurately. Brain uptake is proportional to f_1 , as protein-bound

drug cannot cross the blood–brain barrier. Thus, relatively small changes in protein binding would have significant effects on total brain uptake. For example, if protein binding decreases from 99.9 to 99.8%, then f_1 and brain uptake would increase 100%, from 0.1 to 0.2%. Intersubject variability of baseline distribution volumes in our studies may not reflect actual differences in receptor densities. Instead, it may reflect biological variability in f_1 or our limited accuracy to measure such low values. Future studies should carefully assess methods to accurately measure and assess the impact of f_1 on brain uptake of [¹¹C]MePPEP.

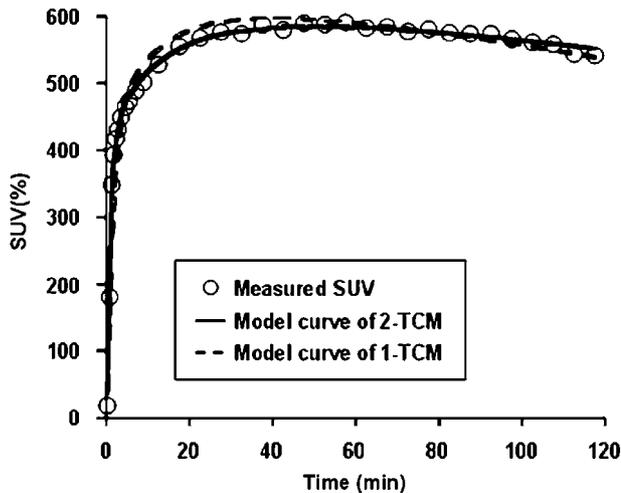


Figure 6 One- and two-tissue compartment analysis. Fitting by two-tissue compartmental model (solid line) was significantly better than that by one-tissue compartmental model (dashed line) for measured %SUV of all regions. The %SUV of all regions was calculated from the volume-weighted average of cerebellum, striatum, prefrontal cortex, lateral temporal cortex, medial temporal region, and pons.

Table 4 Total Distribution Volume of Baseline and Preblocking Study of Two Monkeys

Region	V_T (baseline)	V_T		Average of %
		(preblocking study)	Preblocking/ baseline (%)	
Prefrontal cortex	26.0	2.8	10.9	9.3
	63.3	5.0	7.8	
Lateral temporal cortex	22.4	3.0	13.4	11.6
	54.7	5.3	9.8	
Medial temporal region	19.1	3.0	15.4	13.6
	43.1	5.0	11.7	
Striatum	24.3	2.9	12.1	10.8
	53.8	5.1	9.5	
Thalamus	10.8	3.1	28.2	25.6
	23.3	5.4	23.0	
Cerebellum	21.7	2.5	11.6	10.7
	42.2	4.2	9.9	
Pons	8.6	2.6	30.0	25.3
	21.6	4.4	20.5	

V_T , total distribution volume.

- Although not a limitation specific to [¹¹C]MePPEP, the monkey brain has no region that could be used as reference tissue. We quantified brain uptake relative to concentrations of parent radiotracer in plasma. Reference tissue methods compare uptake in receptor-rich to receptor-free regions within brain and are much simpler to perform. Even the pons, which has a relatively low density of CB₁ receptors, showed significant displacement and blockade (Figure 4b and c). Some of this displaceable activity derived from surrounding regions, because of the limited resolution of the PET camera and the resulting spillover of activity from adjacent tissue. The human brain may be large enough so that a small area like pons could be used as a reference region. Analysis with a reference region would not only avoid arterial plasma sampling but also obviate the need to measure f_1 . That is, normalizing to a reference region effectively corrects for intersubject variations in f_1 .
- [¹¹C]MePPEP may generate radiometabolite(s) that enter monkey brain. The vast majority of brain activity was displaced or preblocked, suggesting that any putative metabolite must retain significant receptor affinity. Nevertheless, additional studies in rodents would be useful to detect the presence and the level of radiometabolites extracted from brain, and any future human studies with [¹¹C]MePPEP should carefully examine the time stability of distribution volume measurements.
- A major concern about [¹¹C]MePPEP is whether humans will have slower brain washout such that distribution volume measurements are unstable within the practicable imaging time of ¹¹C. Scans typically span a total 60–150 min, depending on injected activity and sensitivity of the camera. Brain washout is highly dependent on the peripheral clearance of the radiopharmaceutical, with faster peripheral clearance causing faster brain washout. In the absence of pharmacokinetic data on MePPEP, human studies with tracer doses of [¹¹C]MePPEP are the most expeditious method to determine the utility of the radiotracer. Because of its overall favorable performance in monkeys, we plan to study [¹¹C]MePPEP in humans.

Comparison with Other CB₁ Receptor Radioligands

¹¹C-JHU75528 is an analog of rimonabant and was recently reported as an effective PET probe for CB₁ receptors (Horti *et al*, 2006). As mentioned in the ‘Introduction’, it appears to be the best radioligand published to date for *in vivo* imaging of the CB₁ receptor. ¹¹C-JHU75528 and [¹¹C]MePPEP had similar distribution and time of peak uptake (~20 min) in baboon and rhesus brain, respectively. However, the peak uptake of ¹¹C-JHU75528 may be almost sixfold lower than that of [¹¹C]MePPEP. The brain peak uptake of ¹¹C-JHU75528 is reported to be 0.4% injected activity per 100 ml in putamen in male baboon (26 kg), which corresponds to an SUV of 104%. In contrast, [¹¹C]MePPEP showed peak striatal uptake of ~600% SUV in our study. The percentage-specific binding of these two tracers is difficult to compare, because of different measurements in the two studies. Nevertheless, preblockade decreased striatal uptake of ¹¹C-JHU75528 by about 50%, which is similar to our results with [¹¹C]MePPEP (Figure 4a

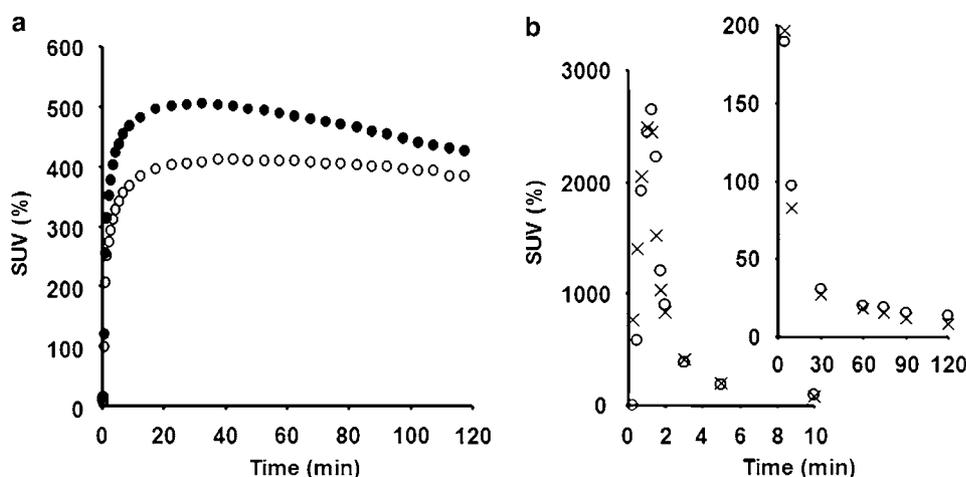


Figure 7 Effect of DCPQ on brain uptake and peripheral clearance of [¹¹C]MePPEP. (a) Average uptake of all measured regions of all animals. DCPQ (8 mg/kg i.v.) increased uptake in all regions. Open circles represent baseline and solid circles DCPQ results. (b) [¹¹C]MePPEP plasma radioactivity, corrected for metabolites, are shown for baseline (O) and P-gp inhibitor (x) studies. Values are total concentration of plasma [¹¹C]MePPEP—free plus protein bound. Left curve shows values from 0 to 10 min; right curve from 5 to 120 min. The curves suggest no significant effect of P-gp inhibition on peripheral clearance.

Table 5 Brain AUC and Plasma-Free Fraction (f_i): Baseline and after DCPQ Administration

Monkey	f_i (%)		Ratio: DCPQ/baseline	
	Baseline	DCPQ	f_i	AUC
D	—	—	—	1.21
E	0.042	0.044	1.05	1.13
F	0.055	0.063	1.15	1.17
Mean \pm SD	0.049 \pm 0.009	0.054 \pm 0.013	1.10 \pm 0.07	1.17 \pm 0.04

AUC, area under the curve.

Brain AUC was calculated for all six regions from 0 to 120 min.

and b). Note that these comparisons of brain time activity curves do not correct for changes in arterial input function, which are required for accurate quantitation of the percentage-specific signal in brain. In brief, [¹¹C]MePPEP and ¹¹C-JHU75528 appear to have fairly similar kinetics of brain uptake and may have similar percentage of specific signal. However, the peak uptake of [¹¹C]MePPEP is about sixfold greater than that of ¹¹C-JHU75528. Both ¹¹C-JHU75528 and [¹¹C]MePPEP are likely to be assessed in humans. Because these two ligands have different structures, species differences in clearance, metabolism, and plasma protein binding may make one tracer clearly superior to the other for human studies.

Conclusion

[¹¹C]MePPEP is promising tracer to measure CB₁ receptors. It readily entered monkey brain, had peak levels within 20 min, and showed relatively stable measurements of distribution volume within 90 min. For quantitation of distribution volumes using an arterial input, the two-tissue compartment analysis was superior to one-tissue for all measured regions. These promising results justify trials of [¹¹C]MePPEP in humans, for which brain uptake should be

carefully analyzed for measuring distribution volume parameters and to provide any evidence of radiometabolites.

ACKNOWLEDGEMENTS

We thank Jonathan P Gourley, BS for help with radiometabolite analysis; Jehi-San Liow for assistance on image analysis; and the staff of the NIH PET Department for successful completion of the scanning studies, and Xenova Group, Ltd (UK) for providing tariquidar. This research was performed via a Cooperative Research and Development Agreement (CRADA) with Eli Lilly, Karolinska Institutet, and NIMH and was supported in part by the Intramural Program of NIMH (project Z01-MH-002795-04). FY was funded by Japanese Society for the Promotion of Sciences, Research Fellow in Biomedical and Behavioral Research at NIH (2005–2006). Eli Lilly provided research grant support to NIMH and the Karolinska Institutet as part of the current CRADA or other research agreements. RBI and VWP (NIMH) have received research support, but no personal income, from Eli Lilly and AstraZeneca. CH (Karolinska Institutet) has received research support and/or personal income from Eli Lilly and AstraZeneca.

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